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Aroma Compound Analysis of *Eruca sativa* (*Brassicaceae*) SPME Headspace Leaf Samples Using GC, GC−MS, and Olfactometry

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The aroma compounds of rocket salad (*Eruca sativa*) SPME headspace samples of fresh leaves were analyzed using GC, GC−MS, and olfactometry. More than 50 constituents of the *Eruca* headspace could be identified to be essential volatiles, responsible for the characteristic intense green; herbal; nutty and almond-like; *Brassicaceae*-like (direction of cabbage, broccoli, and mustard); and horseradish-like aroma of these salad leaves. As aroma impact compounds, especially isothiocyanates, and derivatives of butane, hexane, octane, and nonane were identified. 4-Methylthiobutyl isothiocyanate (14.2%), *cis*-3-hexen-1-ol (11.0%), *cis*-3-hexenyl butanoate (10.8%), 5-methylthiopentyl isothiocyanate (9.3%), *cis*-3-hexenyl 2-methylbutanoate (5.4%), and 5-methylthiopentanenitrile (5.0%) were found in concentrations higher than 5.0% (calculated as % peak area of GC analysis using a nonpolar column).

KEYWORDS: ***Eruca sativa*; fresh leaves; SPME-headspace; aroma compounds; isothiocyanates**

INTRODUCTION

Eruca vesicaria ssp. *sativa* (P. Mill.) Thellung, commonly known as "rucola" in Italy, "Ackerrauke" in Germany, or "arugula" or "garden rocket" in the United States, belongs to the *Brassicaceae* family. This edible herb is used for salad, in sauces, and as a steamed vegetable (*17*). In Asia, the plant serves as an important source of oil seeds (*6*). Garden rocket is an erect, annual herb that ranges from one to two feet tall, although the garden variety can be taller. This species has rough and stiff branched stems. The leaves are very irregular; they are alternate, fleshy, and somewhat hairy, 5−15 cm long. The basal leaves are petiolate (have a stem), and pinnately lobed or divided. They are somewhat pointed, lance-shaped, and deeply indented near the plant base. Leaves found along the stem are smaller. When crushed, the leaves emit a strong odor. The flowers are usually pale, or creamy yellow, with purple veins. The sepals are erect, and the petals taper to a slender claw. The inflorescence is a raceme. The fruit is a silique, with a flattened beak. The seeds are dull yellowish brown, and they are flattened laterally, grooved along one edge (*17*).

The volatiles of plants as well as plant parts from the *Brassicaceae* family using different headspace methods (*4*) and especially of the *E. sativa* leaves responsible for the characteristic odor of this salad plant have been investigated very little. Only a few papers have discussed the aroma compounds of garden rocket leaves (*6, 9*) and the volatiles of the seeds (*1, 15*); all prior research was found by electronic literature search.

To the best of our knowledge no data of aroma compounds of *Eruca sativa* leaves cultivated in Austria were published until now. Therefore, the aim of this work was to trap the volatiles of garden rocket leaves using SPME and to identify the headspace constituents especially responsible for the characteristic green; weak floral-fruity; herbal; weak nutty and almond-like; *Brassicaceae*-like (direction of cabbage, broccoli and mustard); horseradish-like; and weak fatty aroma impression using GC, GC−MS, and olfactometry.

MATERIALS AND METHODS

Plant Material. Rucola (*Eruca sativa*) salad leaves (glass-house cultivar from Austria) were obtained from Delikatessa Co., Wr. Neudorf, Austria in 100-g sealed plastic bags in March 2001 (three bags used) and the botanical identity of the plant material was confirmed by a local botanist.

Sampling Preparation. For SPME sampling, the leaves were cut into 0.2-cm and smaller pieces with a sharp knife and ground to a paste in a mortar and pestle. The paste (100 g) was placed into a 100-mL Erlenmeyer flask, and the flask was closed with a septum. The SPME headspace volatiles were collected using a Supelco 57348 2 cm, 50/30 μm DVB/Carboxen/PDMS Stable-Flex fiber (precondition of the fiber at 250 °C in the injection port of a GC for 4 h) for 12 h (highest concentration of aroma compounds without qualitative change of the composition; tested by 1-h extraction steps up to 24 h). This procedure was done with the content of all three salad bags separately. After sampling, the SPME device was placed into the injector of the GC and the GC−MS instruments through the whole GC analysis time of 38 min using a polar column and 50 min using a nonpolar column, respectively.

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Table 1. SPME Headspace Volatiles of *Eruca sativa* Salad from Austria

compound	KI[a]	conc.[b]	odor[c]
acetaldehyde[e,f,g]	427	0.1	etheral, pungent
acetic acid[e,f,g]	603	0.1	sour
hexanal[e,f]	785	1.1	fatty, green, grassy
tetrahydrothiophene[d,e,f,g]	802	1.2	*Allium*- and cabbage-like
trans-2-hexenal[e,f]	830	0.3	almond-like, green, herbal
furfural[e,f,g]	837	0.8	fresh bread-like, almond-like
cis-3-hexen-1-ol[e,f,g]	849	11.0	fresh, green grass-like
trans-2-hexen-1-ol[e,f]	853	0.7	green, leafy
hexanol[e,f,g]	859	1.6	herbal, mild-woody, sweet
allyl isothiocyanate[d,e,f]	862	0.3	mustard- and horseradish-like
heptan-3-ol[e,f]	877	0.6	herbal
benzaldehyde[e,f,g]	952	1.9	almond-like
3-octanone[e,f]	965	0.2	herbal, buttery
1-octen-3-ol[e,f,g]	968	1.0	champignon-like
hexanoic acid[e,f,g]	971	0.7	fatty, cheesy
octanal[e,f]	975	0.4	fatty, citrus
trans,trans-2,4-heptadienal[d,e,f]	978	1.4	fatty, hazelnut-like
3-octanol[e,f]	981	0.7	mushroom-like, herbal, nutty
2-octanol[e,f]	984	0.5	fatty, oily
2-pentyl furane[e,f]	987	0.8	green bean-like
cis-3-hexenyl acetate[e,f,g]	993	0.6	powerful green
2-methyl anisole[d,e,f]	1001	1.3	warm, floral, walnut-like
5-methylthiopentanenitrile[d,e,f]	1015	5.0	broccoli- and cabbage-like
2-acetyl thiazol[e,f]	1021	1.3	hazelnut-like
limonene[e,f,g]	1027	0.7	citrus, sweet, lemon
benzyl alcohol[e,f,g]	1032	1.2	aromatic, floral
acetophenone[e,f,g]	1041	0.8	sweet, almond-like, floral
nonanal[e,f,g]	1081	0.4	floral, fatty, waxy
trans-2-nonenal[e,f]	1134	1.1	fatty, waxy
nonanol[e,f]	1156	1.4	fatty, green
4-pentenyl isothiocyanate[d,e,f]	1161	3.2	mustard- and horseradish-like
octanoic acid[e,f,g]	1167	0.9	fatty, oily, rancid
cis-3-hexenyl butanoate[e,f]	1178	10.8	green, wine-like
cis-3-hexenyl 2-methylbutanoate[e,f]	1191	5.4	herbal, sweet
β-citronellol[e,f,g]	1211	1.3	rose-like
carveol[e,f,g]	1217	0.4	caraway-like
cumin aldehyde[e,f,g]	1224	0.1	sharp, acid, woody, oily
carvone[d,e,f,g]	1231	0.2	herbal, spearmint/caraway
decanol[e,f,g]	1263	1.0	floral, fatty
nonanoic acid[e,f]	1275	0.8	fatty, cheesy
cuminyl alcohol[e,f]	1284	0.8	floral
undecanal[e,f]	1291	0.2	fatty with floral/fruity notes
dodecanal[e,f]	1396	1.2	herbal, sweet, waxy, floral
methyl eugenol[d,e,f,g]	1407	0.9	spicy, clove-like
β-caryophyllene[e,f]	1418	1.2	spicy, woody, terpene-like
4-methylthiobutyl isothiocyanate[d,e,f]	1447	14.2	cabbage-like
methyl dodecanoate[e,f]	1509	0.1	coconut-like, fatty
δ-cadinene[e,f]	1519	0.1	warm, woody
5-methylthiopentyl isothiocyanate[e,f]	1524	9.3	cabbage-like
hexyl octanoate[e,f]	1566	0.8	vegetable-like, green
dodecanol[e,f,g]	1577	2.2	fatty, waxy, coconut-like
β-farnesol[e,f]	1696	1.7	floral, oily
benzyl benzoate[d,e,f,g]	1723	1.4	sweet, balsamic, nutty
hexadecanol[e,f]	1870	2.2	waxy, floral

[a] Kovats indices using a nonpolar FSOT-RSL-200 column. [b] Concentrations calculated by % peak area of GC–FID analyses using a nonpolar FSOT- RSL-200 column. [c] Odor description using GC–sniffing technique, partly in correlation with published data (*2*, *3*, *10*, *11*, *18*). [d] Reference compound injected. [e] Compound identified by retention time (Kovats index) correlations (GC–FID). [f] Compound identified by mass spectra correlations (GC–MS). [g] Compound identified by olfactoric evaluations (GC–sniffing technique).

Olfactoric Evaluation. The genuine leaves and the paste (total of six samples) were olfactorically evaluated by professional perfumers, and the aroma of both samples was described as the following: green, weak floral-fruity, herbal, weak nutty and almond-like, *Brassicaceae*-like (direction of cabbage, broccoli, and mustard), horseradish-like, and weak fatty in the background.

Instrumentation. Gas chromatographic analyses were performed with a Shimadzu GC-14A with FID and Shimadzu Chromatopac C-R6A integrator, and with a Varian GC-3700 with FID and Shimadzu Chromatopac C-R1B integrator (all Shimadzu, Kyoto, Japan). Compounds were separated on 30 m $\times$ 0.25 mm (i.d.) fused silica columns coated with either a 0.25-μm film bonded nonpolar FSOT-RSL-200 (Bio-Rad, Eke, Belgium), or a 60 m $\times$ 0.25 mm (i.d.; 0.25-μm film) bonded polar Stabilwax (Restek, Bellefonte, PA). The nonpolar column was maintained at 40 °C for 2 min after injection then programmed at 6 °C min^{-1} to 280 °C which was maintained for 10 min (total analysis time 50 min). The temperature program for the polar column was as follows: 40 °C for 2 min up to 250 °C for 10 min with a rate of 10 °C min^{-1} (total analysis time 38 min). Open split injection was conducted with split ratios of 1:20 and 1:50 for the nonpolar and polar columns, respectively; hydrogen was used as carrier gas at 2.5 and 3.5 kPa, respectively. For all columns the injector temperature was 230 °C and the detector temperature was 250 °C (carbowax) or 280 °C (RSL). After analysis on a RSL column, quantification was performed as % peak

area using integration data. Some individual components could be identified by injection of pure compounds and comparison of their retention times (as Kovats indices) with published data (*5*, *8*, *12*, *14*, *19*).

Gas chromatography−olfactometry analysis ('sniffing technique') was performed with a Fractovap 2101 GC equipped with a splitting system, a model 230 LT-Programmer, a model 160 electrometer (Carlo Erba, Milano, Italy), and a Kompensograph-III Recorder (Siemens, Munich, Germany). Compounds were separated on a 30 m × 0.32 mm (i.d.) fused silica column coated with a 0.25-μm film of nonpolar FSOT-RSL-200. The column was maintained 40 °C for 5 min after injection then programmed at 8 °C min^{-1} to 230 °C which was maintained for 20 min. Compounds were injected in splitless mode with hydrogen as carrier gas (pressure 1.8 kPa; column flow 2 mL min^{-1}). The injector temperature was 250 °C, the detector (FID) temperature was 320 °C, and the sniffing capillary temperature was 250 °C. The column eluate sniffing split ratio was 1:50, FID/nose. Peak to odor-impression correlations were performed by two professional perfumers and three fragrance chemists.

GC−MS was performed with a Shimadzu GC-17 gas chromatograph coupled with a Shimadzu QP5000 mass spectrometer (Compaq-Pro Linea data system, class 5k software) and a GC-17A coupled with a QP5050 (Pentium II data system, class 5k software). The columns (FSOT-RSL-200 and Stabilwax) and temperature programs used were the same as those used for GC analysis. Split injection was performed with helium as carrier gas. For the nonpolar column the split ratio was 1:50, the column head pressure was 4.9 kPa, the flow rate was 0.5 mL min^{-1}, the linear velocity was 25.5, and the total flow was 25.6 mL min^{-1}. For the polar column the split ratio was 1:126, the head pressure was 115.5 kPa, the flow rate was 1.0 mL min^{-1}, the linear velocity was 26.0, and the total flow was 131.1 mL min^{-1}. Injector, interface, and ion-source temperatures were 230, 250, and 200 °C, respectively. The spectrometers were operated in electron-impact (EI) mode with 1.2 kV detection volts; the scan range was 41−400 amu; the scan interval was 0.50 s, and the scan speed was 1000 amu sec^{-1}. Compounds were identified by use of NIST, Wiley, NBS, and our own mass spectra libraries, as well as literature MS data (*7*, *12*, *13*, *16*).

RESULTS AND DISCUSSION

The genuine *Eruca sativa* salad leaves, cultivated in Austria, and the corresponding prepared paste samples were evaluated by professional perfumers for their characteristic aroma as already described above. The intensity of the odor of the rucola paste samples was higher, by a factor of about 100, than that of the genuine leaves (determined by odor evaluations of professional perfumers). No difference was found in the quality of the aroma impression between *E. sativa* leaves and the corresponding paste sample. Therefore, the volatiles of paste samples were trapped by SPME and analyzed by GC−FID and GC−MS using columns of different polarities to identify the aroma target compounds of this natural product.

In total, 54 constituents of the *Eruca sativa* SPME headspace (leaves) could be identified among the more than 60 components detected by GC−FID and GC−MS (Kovats indices and mass spectra correlations; **Table 1**). As main compounds (concentration higher than 5.0%, calculated as % peak area using GC−FID with a nonpolar FSOT-RSL-200 column) the following were found: 4-methylthiobutyl isothiocyanate (14.2%), *cis*-3-hexen-1-ol (11.0%), *cis*-3-hexenyl butanoate (10.8%), 5-methylthiopentyl isothiocyanate (9.3%), *cis*-3-hexenyl 2-methylbutanoate (5.4%), and 5-methylthiopentanenitrile (5.0%). Further, more than 45 volatiles with dominating butane, hexane, octane. and nonane structures were identified. All these *E. sativa* SPME headspace constituents have been described elsewhere (*2*, *3*, *10*, *11*, *18*) to possess a characteristic aroma. Therefore, GC−olfactometry ('GC−sniffing technique') was used to correlate the single aroma impression from the GC eluate with the gas chromatographic and spectroscopic data.

This chromatographic−olfactoric result of identified volatile compounds of *Eruca sativa* is in agreement with the published odor attributes for each single SPME headspace compound and allows the following statements. (1) For the green aroma impression of the investigated Austrian *Eruca sativa* salad, hexanal, *trans*-2-hexenal, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, 2-pentyl-furane, *cis*-3-hexenyl acetate, nonanol, *cis*-3-hexenyl butyrate, and hexyl octanoate are responsible. (2) Floral-fruity odor notes can be attributed to limonene, acetophenone, nonanal, β-citronellol, decanol, and undecanal. (3) Herbal aromas are caused by hexanol, *trans*-2-hexenal, heptan-3-ol, 3-octanone, 3-octanol, benzyl alcohol, *cis*-3-hexenyl 3-methylbutanoate, carvone, and dodecanal. (4) Nutty and almond-like odor impressions are known from furfural, benzaldehyde, *trans*,*trans*-2,4-heptadienal, 3-octanol, 2-methyl anisole, 2-acetyl thiazole, acetophenone, methyl dodecanoate, dodecanol, and benzyl benzoate. (5) The *Brassicaceae* (direction of cabbage, broccoli, and mustard) aroma can be correlated to tetrahydrothiophene, allyl isothiocyanate, 5-methylthiopentanenitrile, 4-pentenyl isothiocyanate, 4-methylthiobutyl isothiocyanate, and 5-methylthiopentyl isothiocyanate. (6) Allyl isothiocyanate and 4-pentenyl isothiocyanate are responsible for the horseradish aroma. (7) Hexanal, hexanoic acid, octanal, *trans*,*trans*-2,4-heptadienal, 2-octanol, nonanal, *trans*-2-nonenal, nonanol, octanoic acid, decanol, nonanoic acid, undecanal, methyl dodecanoate, dodecanol, and hexadecanol compose the fatty side-notes of this *Eruca sativa* from Austria.

In summary, the significant aroma of *E. sativa* leaves is not the result of one single odor impression. The combined use of SPME headspace analysis with GC−FID, GC−MS, GC−sniffing technique and olfactometry led to the identification of 54 characteristic aroma impact compounds of *E. sativa*. Several isothiocyanates and numerous butane, hexane, octane, and nonane derivatives were found to be of essential importance for the characteristic aroma of this food plant.

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